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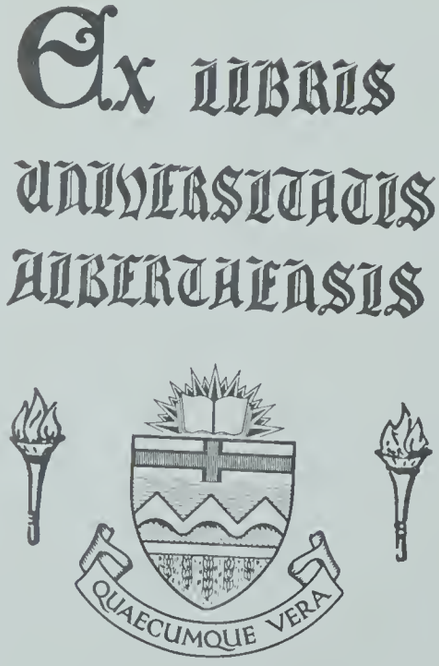

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THE UNIVERSITY OF ALBERTA

PREDICTION OF METABOLIZABLE ENERGY CONTENT OF FEEDSTUFFS AND RATIONS FOR BROILER CHICKENS

by

© ALAN JAMES LESLIE

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Prediction of Metabolizable Energy Content of Feedstuffs and Rations for Broiler Chickens" submitted by Alan James Leslie, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Experiments were conducted to assess the degree of accuracy with which metabolizable energy content of rations and feed ingredients could be predicted from their chemical analyses. Feeding trials were then conducted to study the substitution of grains in nutrient-equivalent broiler rations using biologically determined as well as predicted metabolizable energy values for the substitution.

In order to ascertain the accuracy of predicted values, the metabolizable energy content of 14 rations and 6 feed ingredients was determined using a biological assay procedure and chemical analyses for protein, ether extract and available carbohydrate were used to predict their metabolizable energy content. Two methods were used to measure available carbohydrate, one employing Fehlings reagent and the other Glucostat reagent. These resulted in different estimations of available carbohydrate. When the predicted values were compared to biologically determined values, correlation coefficients for rations, ingredients and the combination of both were 0.87, 0.79 and 0.81 respectively when available carbohydrate was determined using Fehlings reagent, and 0.75, 0.72 and 0.71 respectively when Glucostat reagent was used. Separate prediction equations were also derived for rations, feed ingredients and the combination of rations and feed ingredients for each method of analysis used. Although there was an indication that prediction of metabolizable energy was more accurate when available carbohydrate was determined using Fehlings reagent, the procedure involving

Glucostat reagent was more rapid and thus was adopted as being the more practical method.

The results of feeding trials with broilers indicated that it was possible to replace the grain portion of the ration with wheat, oats, barley or combinations of the grains without affecting rate of growth, provided the substitution was made on a nutrient-equivalent basis. The substitution had no effect on efficiency of feed conversion except when barley was used as the only grain in the ration in which case efficiency of feed conversion was reduced. Predicted values for metabolizable energy appeared to be equivalent to determined values for formulation of isocaloric rations.

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INTRODUCTION

Improvements in efficiency of poultry production over the past several decades has resulted in chicken being among the least expensive meat products available. Although this improvement would not have been possible without advances in the field of genetics, marked progress in nutrition was also necessary. The introduction of high-energy rations and recognition of the fact that chickens tend to eat to meet their energy requirements has made it necessary to relate the levels of other nutrients to the energy level of the ration. This has resulted in the nutritionist being able to adjust formulations to meet the nutrient requirements of the class of poultry being fed.

The most limiting factor in formulating rations to precise nutrient composition has been a lack of accurate knowledge of the metabolizable energy content of feed ingredients. Although fairly simple chemical analyses exist for measurement of protein and fat, measurement of metabolizable energy content of a ration or feed ingredient requires a biological determination which is expensive and time-consuming. If a simple method were available to measure or predict the metabolizable energy content of a feedstuff, accurate formulation of rations would be easier. Consequently experiments were designed to determine the accuracy with which metabolizable energy content of feedstuffs could be predicted and the effect on the performance of chickens of feeding rations that were formulated using determined and predicted metabolizable energy value.

REVIEW OF LITERATURE

History

The first experiments on the role of energy in poultry nutrition were stimulated by the studies of Fraps (1946) which indicated a wide variation in the productive energy content of poultry feed ingredients. The more fibrous feeds tended to be lower in energy content.

Chick performance was soon found to be markedly affected by the energy level of the ration. Scott, Matterson and Singsen (1947) developed a high energy broiler ration by substituting wheat and corn for more fibrous ingredients in the ration such as oats and by-products of wheat milling. The high energy ration resulted in increased rate of growth and improved feed efficiency. It was observed that fibre per se was not required for the physiological well being of the chick.

It had been noted previously that chick performance was affected by the fibre content of the ration. Heuser et al. (1944) observed that the more fibrous feeds such as oats and wheat by-products were less efficient for growth and maintenance of body weight than less fibrous feeds such as wheat, corn and rolled oats. Following the discovery of the efficacy of high energy rations (Scott et al., 1947) other workers demonstrated that performance was affected by the fibre content of the ration. Panda and Combs (1950) concluded that the growth depressing effect of additional crude fibre from wheat, wheat bran, wheat standard middlings, dehydrated alfalfa meal, pulverized heavy oats or ground corn cobs, replacing corn in a ration, was due to their lower productive energy content. Dansky and Hill (1951) observed that

growth rate was reduced by addition of high levels of cellophane, sugar cane pulp or cellulose to a ration in inverse proportion to the bulk of the ration.

In a short time it became apparent that energy level of rations exerted a profound influence on the performance of chicks. Hill and Dansky (1954) observed that as energy level of a ration was reduced by addition of oat hulls, at constant protein levels, feed intake increased and feed efficiency was reduced but rate of gain was not affected. It was also found that as protein levels were decreased, at constant energy levels, growth rate was reduced but feed consumption was not affected. Peterson, Grau and Peek (1954) noted that the need for energy was the primary factor controlling voluntary feed intake in young chickens and suggested that chickens eat to meet their energy requirements. This was corroborated by Fisher and Weiss (1956). Dansky and Hill (1951) had previously demonstrated the chicks remarkable ability to compensate for reduced dietary energy by increasing feed consumption within physiological limits.

Subsequently it was recognized that although an inverse relationship usually existed between the fibre level of a ration and its energy content, the two factors were not necessarily interdependent. Richardson, Watts and Epps (1958) noted that additional fibre from rice hulls reduced feed efficiency although it did not affect weight gain. The effect of the additional fibre on feed efficiency could be nullified by supplementing the ration with fat. Level of productive energy in the ration was found to be highly correlated with feed efficiency and a better criterion of the worth of a ration than either ration or caloric density. This disagreed with work conducted by Mraz, Boucher

and McCartney (1957) and corroborated by Sibbald, Slinger and Ashton (1960) indicating that the best criterion for measuring the adequacy of a ration was the ratio of the energy content of the ration to its volume rather than to its weight.

The importance of maintaining a relationship between energy and protein content of a poultry ration was soon recognized. Combs and Romoser (1955) first introduced the term "calorie:protein ratio". They suggested that, for maximum performance of chicks, a fixed ratio should be maintained between the productive energy and the protein content of a ration. Hill and Dansky (1950) had previously found that chick growth was reduced when a high energy, low protein ration was fed but when the energy level was reduced, growth improved. Sunde (1956) demonstrated that a high protein, low energy ration reduced growth rate and feed efficiency, but increasing the energy level improved both factors. Matterson et al. (1955) observed that as the productive energy level was varied, at any given protein level from 20 to 28 per cent, the calories consumed per unit of gain remained approximately the same. Donaldson, Combs and Romoser (1956) indicated that the productive energy level of a ration influenced the level of protein required in the ration for optimum growth and feed efficiency of the chick. It was noted that, as the calorie:protein ratio widened, energy intake and carcass fat content increased; the birds apparently overconsumed energy to satisfy their requirements for other limiting nutrients, presumably amino acids.

Metabolizable Energy

Recognition of the importance of energy level of poultry

rations eventually led to the assessment of the suitability of different measures of energy for use in poultry nutrition. Initially, productive energy was considered to be the most satisfactory measure of energy in feeds. Fraps and Carlyle (1939) concluded that metabolizable energy (M.E.) did not correctly measure the value of a feed for growth and production. Later Fraps (1946) reported results of extensive studies on the productive energy content of feeds and feed ingredients. The availability of the values enabled investigators to apply them to their studies and it was recognized that efficiency of production was closely related to the productive energy content of the ration fed (Dymsza, Boucher and McCartney, 1955; Hill, Anderson and Dansky, 1956).

It was only a short time, however, before investigations were again undertaken to study the use of M.E. values of feeds in poultry nutrition. This led to experiments designed to assess the relative merit of using metabolizable or productive energy as a measure of energy in nutritional work. Davidson, McDonald and Williams (1957) concluded that the productive energy values of Fraps (1946) were unreliable due to inconsistencies in the algebraic calculations involved. Anderson, Hill and Renner (1958), Hill and Anderson (1958) and Potter et al. (1960) observed that M.E. values were a more precise measure of energy than productive energy values because of the greater degree of variability that existed in the measurement of productive energy. This was confirmed by Vohra (1966) who concluded that M.E. was a better indicator of nutritive value of a ration than net energy.

Adoption of metabolizable energy as the preferred measure of energy in poultry nutrition led to investigation of factors affecting

M.E. values. Some factors were found to affect M.E. values but most had little effect. Baldini (1960) indicated that the M.E. level of a methionine-deficient ration was higher than that of a balanced ration. Although Carew and Hill (1961) could find no effect of a methionine deficiency on M.E., Sibbald, Slinger and Pepper (1962), in studies of the interrelationships among riboflavin, niacin, vitamin B_{12} and methionine, observed that the effect of methionine on M.E. depended on the level of other supplements used. In subsequent work Sibbald and Slinger (1963c) found no effect of inadequacies or excesses of amino acids on the M.E. content of a ration. Olson et al. (1961) noted a significant decrease in the M.E. content of meat meal as the level of inclusion of meat meal in the ration increased but a similar effect could not be demonstrated with soybean meal. Sibbald and Slinger (1962a) reported that level of inclusion of high protein feedstuffs in a ration had no effect on the M.E. content of the feedstuff. Sibbald, Summers and Slinger (1959) and Sibbald, Summers and Slinger (1960) observed that while the M.E. content of corn differed significantly depending on the other components of the ration, that for wheat did not.

Age of bird and species have been found to affect the level of M.E. in rations. Sibbald et al. (1959) demonstrated that the M.E. content of several feedstuffs differed when chicks of 3 or 7 weeks of age were used for the determinations, although these findings could not be confirmed the next year (Sibbald et al., 1960). Zelenka (1968) also reported that the M.E. content of rations varied as the birds aged. Slinger, Sibbald and Pepper (1964) indicated that differences existed between chickens and turkeys in their ability to metabolize

energy from either high or low energy rations. These differences were later confirmed by Begin (1967) and Foster (1968).

Biological Determination of Metabolizable Energy

Several different methods have been used by different research groups for the determination of M.E. in poultry feedstuffs. The methods in most common use are those of Hill et al. (1960) and Sibbald and Slinger (1963b). The method used by Hill et al. (1960) consisted of feeding a semi-purified basal ration containing a reference material along with a test ration similar to the basal ration but with a portion of the reference material being replaced by the ingredient to be assayed. The M.E. content of the test ingredient was calculated from the M.E. content of the two rations fed. The method of M.E. determination used by Sibbald and Slinger (1963b) consisted of feeding a basal ration composed of natural ingredients and a test ration formulated by replacing part of the basal ration with the material to be assayed. By determining the M.E. content of both rations, the M.E. content of the test material could be calculated.

Some comparisons have been made between the two methods of M.E. determinations and it appears that each has advantages and disadvantages. The method of Hill et al. (1960) assumed that the M.E. value of the reference material remained constant regardless of age, type of chick or feedstuff being assayed; assumptions that have been questioned by several research workers (Sibbald et al., 1959; Slinger et al., 1964; Zelenka, 1968). The above assumptions were not necessary when the method of Sibbald and Slinger (1963b) was used

since an internal control was included in each set of determinations. Recently, Renner[1] suggested that the rations used by Sibbald and Slinger (1963b) may have been nutritionally imbalanced since large portions of the basal were removed and replaced with a single ingredient. Cullen, Rasmussen and Wilder (1962) reported that M.E. values for single ingredients differed depending on whether semi-purified or natural rations were used in the determinations.

In M.E. determinations a correction for tissue storage of protein is usually made. Hill and Anderson (1958) suggested a method for correction that has been used widely. The methods of M.E. determination most commonly used (Hill et al., 1960 and Sibbald and Slinger, 1963b) both used this correction factor. Although it has been suggested (Baldini, 1961; Sibbald and Slinger, 1962c) that a correction for nitrogen retention was not necessary, variations in nitrogen retention (Slinger et al., 1964) appeared to indicate that use of a correction factor was desirable.

Most of the M.E. values reported in the literature have been derived by the methods of Hill et al. (1960) and Sibbald and Slinger (1963b) and, despite the differences in the determinations, there is fairly close agreement among the values determined. Several investigators (Anderson and Hill, 1955; Potter et al., 1962; Lockhart, Bryant and Bolin, 1966) found that the M.E. content of feedstuffs was not affected significantly by the methods of determination used although Kalmbach and Potter (1959) did find differences. Sibbald and Slinger (1962a) concluded that the method of calculation could have

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[1] Renner, R. Personal communication.

a significant effect on the M.E. value derived for a feedstuff. It was observed that the reliability of the M.E. values obtained increased when more than one level of the test material was used in the determination.

Prediction of Metabolizable Energy from Chemical Analysis of Feedstuffs

Energy in a feedstuff is derived principally from its carbohydrate, protein and fat components. The levels of the components may vary from one sample to another to such an extent that the accuracy of using average energy values for any particular sample of feed is often doubtful. Hoppner, Owen and Sosulski (1968) demonstrated a wide variation in the proximate principles of feed grains between varieties and from year to year. The wide range of M.E. values for poultry feedstuffs reported by Sibbald and Slinger (1962b) and Matterson et al. (1965) indicated a need for M.E. values based on the particular feed ingredient involved. It was therefore logical that attempts be made to derive M.E. values by conducting chemical analyses for the components involved.

The relationship between the analysis of a feedstuff and its feeding value has been of interest for many years. Henneberg and Stohmann (1860) divided the carbohydrate complex into crude fibre and nitrogen-free extractives (N.F.E.) and considered N.F.E. to be a reasonably accurate measure of digestible carbohydrate for the ruminant. Although this designation was applicable only to ruminants it was in general use for many years. Subsequently the Medical Research Council on human nutrition in Britain in 1945 adopted a new method of evaluation of carbohydrates. The total amount of starch,

dextrin, di- and mono-saccharides was determined, expressed in terms of starch, and designated as "available carbohydrate". This term assumed that starch and sugar were completely digestible and that other carbohydrates were completely indigestible. The procedure took into account the fact that the carbohydrate complex was composed of several components not all of which made a contribution to the M.E. content. This was later corroborated by Bolton (1954). Using a similar carbohydrate analysis in conjunction with an analysis for proximate principles, Halnan (1951) was able to derive coefficients of energy yield of the components and to predict the M.E. content of feedstuffs. Using only standard proximate analysis, corrected by digestibility coefficients and multiplied by energy yield coefficients, Axelsson and Eriksson (1951) were also able to predict M.E. values with reasonable accuracy.

Since a portion of the energy content of most feedstuffs comes from the available carbohydrate component, the level of available carbohydrate must be accurately estimated to yield reliable M.E. values from a prediction equation. Clegg (1956) developed an analysis for available carbohydrate based on the colorimetric analysis of starch and sugar using Anthrone reagent. Using the above procedure, Carpenter and Clegg (1956) derived equations for the prediction of M.E. from the chemical analysis of protein, available carbohydrate and ether extract. Davidson et al. (1961), Sibbald et al. (1962) and Sibbald et al. (1963) later used methods similar to those of Carpenter and Clegg (1956) and obtained close agreement between analytically and biologically determined M.E. values. Bolton (1960) developed an enzyme-based analysis for measuring available carbohydrate which yielded results similar to those

of Carpenter and Clegg (1956) but which had the advantage that the determination could be made more rapidly. In this method glucose released by the enzyme was measured quantitatively using Fehlings reagent (AOAC, 1960).

Substitution of Cereal Grains in Rations for Chickens

Studies on the feasibility of using different grains in poultry rations have given results that are often difficult to assess. Since cereal grains contribute different levels of protein and amino acids and also differ in their energy content, the effect of one factor may obscure the results obtained when studying the others. Maw (1939) observed that a corn-based ration resulted in significantly more fat deposition than other grains with wheat, oats and barley following in that order. Biely et al. (1951) found that wheat could be substituted for 75 to 100% of the corn in a high energy broiler ration on a weight-equivalent basis without affecting weight gains. When the protein level was adjusted to compensate for the higher levels present in wheat, weight gains were reduced. Davidson, Mathieson and Williams (1962) also found that weight gains of chicks were affected when grains were substituted in a ration on a nutrient-equivalent basis. Since the protein content of the rations was limiting, the grain with the best amino acid balance, in this case oats, outperformed the others. This confirmed work by Carpenter and Clegg (1957) who had previously observed that oats contained higher quality protein than barley. Sibbald and Slinger (1963a) observed that, on a weight-equivalent basis, wheat supported highest weight gains followed by barley and then oats. This was in the same decreasing order as M.E.

content and protein level in the grains.

Despite the fact that substitution of grains in poultry rations has given variable results, some evidence has indicated that, within the birds ability to consume feed, the type of grain used had no effect on performance provided adjustments were made to make the rations equivalent on a nutrient basis. When Waldroup et al. (1967) compared corn, wheat and milo for turkeys on a weight-equivalent basis, significant differences were found in performance but when the same grains were compared on a nutrient-equivalent basis, using linear programming, there were no differences. Ozment et al. (1962) had previously demonstrated no difference in performance between four types of milo and one type of corn in rations formulated to be of equal nutritional value.

It has been reported that performance of chicks fed a high level of barley in the ration may differ from that which might be expected on the basis of the nutrient content of the ration. Many attempts have been made to discover the reason for this difference. Fry et al. (1958) indicated that the carbohydrate portion of barley may be partially unavailable to the chick. Arscott, Rose and Harper (1960) postulated that barley contains an inhibitor which does not allow the chick to make maximum use of the grain. Although treatment of the barley with water or certain enzymes reduced the negative effect, the way in which this was accomplished was not known.

EXPERIMENTS AT THE UNIVERSITY OF ALBERTA

Experiments were designed to study:

I. Prediction of metabolizable energy of feeds from chemical analyses.

II. Substitution of cereal grains in chicken rations on a nutrient-equivalent basis.

I. Prediction of Metabolizable Energy of Feeds from Chemical Analyses

Status of the Problem

Formulators of poultry feeds use relatively inexact average analyses values for feed ingredients in calculating metabolizable energy levels of rations. Since M.E. is of primary importance in determining the productive performance of a ration (See Review of Literature), the use of average values may result in a reduction in the accuracy with which rations of highest economic efficiency can be formulated. While several methods for the prediction of M.E. based on the chemical analysis of the feedstuff have been advanced, they have generally been too inaccurate or too cumbersome to use under practical conditions. It would therefore be of considerable practical value if a simple, accurate method were available that would permit prediction of M.E. content of a feedstuff from chemical analysis of the feed. The following experiment was undertaken to determine the relationship between metabolizable energy values predicted from chemical analysis and those determined using a chick bioassay.

Experimental

A. Biological determination of metabolizable energy values.

Six hundred, day-old, crossbred (Dominant White × White Plymouth

Rock) male chicks were placed in electrically heated batteries with raised screen floors and were fed a high quality starting ration. The chicks were weighed at 1 and 2 weeks of age. At two weeks of age, 420 chicks were selected for the experiment on the basis of their growth potential and weight and were divided into 28 comparable groups of 15 birds each. Two groups were placed on each experimental treatment. Feed and water were supplied ad libitum. The chicks were fed the experimental ration for 14 days at which time the experiment was terminated.

Metabolizable energy of feed ingredients and rations was determined using a method similar to that of Sibbald and Slinger (1963b) as outlined in Appendix A. The basal rations used (Table 1) in this experiment were composed of natural feed ingredients. Basal 1 was used for assay of low protein ingredients such as cereal grains. Basal 2 was used for the determination of M.E. in higher protein feedstuffs.

Table 1. Composition of basal rations

Ingredients	Basal 1 %	Basal 2 %
Ground wheat	7	40
Ground corn	20	40
Dehydrated alfalfa meal	4	4
Stabilized animal tallow	5	5
Meat meal (55% protein)	10	--
Soybean meal (44% protein)	50	9
Herring meal (72% protein)	4	2

The mixtures used to form the assay rations which comprised the treatments for this experiment are shown in Table 2. Rations for determining M.E. of each grain were composed by substituting 50 and 60 per cent of the grain for Basal 1; rations for determining M.E. of each protein supplement were composed by substituting 25 and 40 per cent of the supplement for Basal 2. A vitamin - mineral supplement (Table 3) was added at a constant level (4.135%) to each assay ration. No attempt was made to standardize the protein levels of the assay rations as it had been previously demonstrated that protein level of the ration had no effect on the metabolizable energy content of feeds (Sibbald and Slinger, 1962a).

B. Prediction of metabolizable energy values from chemical analysis

All samples to be analysed were ground to pass a 20 mesh screen and were analyzed for protein, ether extract and available carbohydrate content. Protein (N × 6.25) was determined by the method of Kjeldahl (AOAC, 1960). Ether extract was determined by the method outlined in AOAC (1960). Two methods were used to estimate the level of available carbohydrate in rations and feed ingredients. The first method used was that of Bolton (1960) in which Fehlings reagent (AOAC, 1960) was used to estimate the level of available carbohydrate. The second method used was identical to the first except that Glucostat reagent was used to estimate available carbohydrate. Details of the analyses for available carbohydrate are given in Appendix B.

The M.E. values of rations and test ingredients were calculated

Table 2. Mixtures used in assay rations

Assay ration no.	Mixtures used in assay rations			
	Basal ration	%	Test ingredient	%
1	1	100	--	0
2	2	100	--	0
3	1	50	Wheat	50
4	1	40	Wheat	60
5	1	50	Oats	50
6	1	40	Oats	60
7	1	50	Barley	50
8	1	40	Barley	60
9	2	75	Meat meal	25
10	2	60	Meat meal	40
11	2	75	Herring meal	25
12	2	60	Herring meal	40
13	2	75	Soybean meal	25
14	2	60	Soybean meal	40

Table 3. Composition of vitamin-mineral premix[1]

Ingredients	g
Manganese sulphate	25.08
Zinc oxide	10.12
Dicalcium phosphate	998.8
Ground limestone	800.8
Iodized salt	501.6
Chromium sesquioxide	299.2
Vitamin A (10,000 IU/g)	49.94
Vitamin D_3 (750,000 ICU/g)	99.88
Vitamin E (20,000 IU/g)	49.94
Menadione sodium bisulphite	0.22
Calcium pantothenate	2.20
Riboflavin	0.66
Niacin	4.40
Choline chloride	37.4
Vitamin B_{12}	0.0013
Folic acid	0.22
DL-methionine	49.94
Penicillin G	0.88
Shorts	1203.7187

[1]Amounts added per 100 kg of assay ration.

from the analytical data obtained using the following prediction equation derived by Carpenter and Clegg (1956):

M.E. per g of ration = 0.053 + 0.038 (% protein + 2.25 × % ether extract + 1.1 × % starch + % sugar) .

The equation was modified by substituting available carbohydrate determined by the method of Bolton (1960) for the value "1.1 × % starch + % sugar" which was used by Carpenter and Clegg (1956). Thus, the prediction equation used in this experiment was as follows:

M.E. per g ration = 0.053 + 0.038 (% protein + % available carbohydrate + 2.25 × % ether extract) .

C. Statistical analysis.

The metabolizable energy values of the rations and the feed ingredients obtained from chemical analysis were compared with values determined by chick bioassay by means of correlation and regression analysis. The analyses were performed on an IBM/360 model 67 computer using program REG (Smillie, 1969).

The combined levels of protein, available carbohydrate and ether extract for each ration and test ingredient,expressed as "total analysis" was regressed onto the biologically determined M.E. values. The resulting regression equations expressed the mathematical relationship between the total chemical analysis and the M.E. content of the rations and feed ingredients in this experiment and were later used as prediction equations.

Results and Discussion

The results of chemical analyses performed on mixed rations and feed ingredients (Table 4) indicated that the method of carbohydrate analysis used affected the values obtained. Analysis of available carbohydrate using Fehlings reagent (FCA) gave higher values, in all cases but one, than were obtained when the Glucostat carbohydrate analysis (GCA) was used. Since the results of the carbohydrate analysis using Fehlings reagent compared favorably with those reported by Carpenter and Clegg (1956), it would appear that the GCA underestimated the available carbohydrate content of the materials analyzed. No explanation for the higher values that were obtained for oats using GCA can be advanced.

It is difficult to suggest a reason for the higher levels of available carbohydrate obtained when Fehlings reagent was used as compared to GCA. The procedure followed in the analyses was the same for both methods of measurement to the stage at which glucose was to be measured. Since GCA measures β-d-glucose specifically it would appear that either FCA was measuring other reducing sugars as well as β-d-glucose or that a part of the glucose was not in the form of the β-d-isomer. The possibility exists that some of the glucose may have isomerized during the preparation of the samples and consequently was not measured by GCA. It may therefore be assumed that FCA yielded a more accurate measure of available carbohydrate than did GCA, under the conditions of the experiment.

Metabolizable energy values of rations and feed ingredients determined biologically or calculated from chemical analysis are

Table 4. Analysis of rations and feed ingredients used in Experiment 1[1]

Samples	Crude protein	Ether extract	Available carbohydrate (Fehlings)	Available carbohydrate (Glucostat)
	%	%	%	%
Ration				
1	34.95	7.97	31.07	19.48
2	16.21	8.32	54.34	43.78
3	24.13	5.22	45.59	37.38
4	22.21	4.72	49.24	41.97
5	24.06	7.18	39.60	29.11
6	21.80	7.05	41.60	28.00
7	24.14	4.94	42.69	31.23
8	21.55	4.69	46.78	37.92
9	26.03	8.94	41.65	29.45
10	32.51	9.41	33.96	27.57
11	31.37	8.57	41.34	30.83
12	40.50	8.38	30.56	17.44
13	24.06	6.42	45.75	36.79
14	23.84	6.00	45.37	35.22
Feed Ingredients				
Wheat	14.44	2.12	69.11	58.60
Oats	11.72	6.09	46.13	48.38
Barley	11.34	1.94	61.07	48.10
Meat meal	57.93	11.25	--	--
Herring meal	76.37	9.46	--	--
Soybean meal	48.18	0.99	22.31	5.93

[1]All results reported on a dry-matter basis.

presented in Table 5. For purposes of comparison and statistical analysis the biologically determined values were considered to be the actual M.E. values. Comparable values taken from tables of average analyses used routinely at the University of Alberta are also shown.

Correlation coefficients (r_{xy}, Table 6) indicated a closer relationship between biologically determined M.E. values and FCA values than between biologically determined M.E. values and either GCA values or values based on tables of average analysis. Calculation of coefficient of determination (r^2, Table 6) showed that the FCA-based prediction of M.E. accounted for 16% more of the variability of the components contributing to M.E. content than did the GCA-based prediction. Although M.E. values from tables of average analysis appeared to be as accurate as GCA-based predictions of M.E. in the case of mixed rations, they were less accurate than GCA-based values for individual feed ingredients. On the basis of the above observations it would appear that M.E. values derived from FCA-based predictions were more closely related to biologically determined values than GCA-based predictions or values in the table of average analysis.

A regression equation showing the relationship between actual M.E. value (Y) and the predicted M.E. value (X) of a feedstuff, assuming that all of the energy in the sample was derived from the components being measured and the components were measured with absolute accuracy, would have the formula:

$$Y = 0 + 1(X)$$

The regression coefficients (b_{yx}) determined for this experiment

(Table 6; Figure 1) indicated that M.E. values predicted using FCA as one of the components closely approached this ideal. The GCA-based analysis yielded regression coefficients (Table 6; Figure 2) which were less ideal. It appeared (Figure 2) that meat meal was a major factor contributing to the inaccuracy of the GCA values but when the regression analysis was recalculated excluding meat meal the apparent accuracy of the prediction was not improved. M.E. values of ingredients or rations taken from tables of average analyses regressed onto biologically determined M.E. values demonstrated that a very limited relationship existed between average and determined values.

Calculation of Standard Errors of Estimate and F values (Table 6) indicated that the FCA-based M.E. values gave a higher degree of precision and significance than GCA-based values or use of average analysis values.

Regression coefficients (Table 7) were calculated from the regression of the total chemical analysis of a sample according to the formula X = % protein + % available carbohydrate + 2.25 × % ether extract, on biologically determined M.E. values. The regression equations were plotted in Figures 3 and 4. The values indicated that the FCA-based analysis yielded similar prediction equations for both rations and single ingredients whereas those for GCA-based equations were relatively divergent. The equation based on FCA data more closely approximated the original equation derived by Carpenter and Clegg (1956). Complete agreement with the original equation probably could not be expected since different analyses for carbohydrate were used. Sibbald et al.(1963) obtained very close agreement with the above equation when the same analytical technique was used.

Table 5. A comparison of biologically determined and predicted metabolizable energy values of rations and feedstuffs[1]

Assay material	Determined values	Predicted values		
		F.C.A.[2]	G.C.A.[3]	Average values[4]
Rations	(kcal/g)	(kcal/g)	(kcal/g)	(kcal/g)
1	2.965	3.243	2.803	3.197
2	3.330	3.445	3.044	3.670
3	3.057	3.149	2.837	3.287
4	3.051	3.172	2.895	3.317
5	2.821	3.086	2.688	3.083
6	2.850	3.065	2.548	3.040
7	2.916	3.015	2.580	3.209
8	2.940	3.050	2.714	3.213
9	3.122	3.389	2.926	3.267
10	3.020	3.383	3.141	3.027
11	3.509	3.549	3.149	3.539
12	3.454	3.470	2.971	3.457
13	3.040	3.255	2.914	3.463
14	3.130	3.196	2.810	3.329
Feed ingredients				
Wheat	3.237	3.409	3.010	3.416
Oats	2.970	2.772	2.857	2.970
Barley	3.105	2.971	2.478	3.271
Meat meal	2.715	3.216	3.216	2.173
Herring meal	3.773	3.764	3.764	3.140
Soybean meal	2.600	2.816	2.194	2.502

[1]All results reported on a dry matter basis.

[2]F.C.A. - Fehlings carbohydrate analysis.

[3]G.C.A. - Glucostat carbohydrate analysis.

[4]Average values - taken from tables of average analyses.
A.R. Robblee - personal communication.

Table 6. Correlation and regression analysis between predicted and determined metabolizable energy values for rations and feed ingredients in Experiment 1.

Analysis[1]	r_{xy}	r^2	a	b_{yx}	S.E.E.	F
Fehlings: rations	0.87	0.76	-0.3542	1.0593	0.1083	37.1914**
Fehlings: ingredients	0.79	0.63	0.3145	0.8715	0.2839	6.9237
Fehlings: combination	0.81	0.66	0.1318	0.9154	0.1648	35.6906**
Glucostat: rations	0.75	0.56	0.6622	0.8479	0.1452	15.3553**
Glucostat: ingredients	0.72	0.52	1.4772	0.5444	0.3261	4.2754
Glucostat: combination	0.71	0.50	1.3371	0.6059	0.2005	18.2586**
Average: rations	0.81	0.66	0.1031	0.9059	0.1281	23.1478**
Average: ingredients	0.70	0.49	1.2821	0.6128	0.3344	3.8699
Average: combination	0.64	0.41	1.4275	0.5200	0.2182	12.6302**

[1]All analyses are compared statistically with the biologically determined metabolizable energy values.

**Statisically significant regression equation ($P < 0.01$) .

Table 7. Regression of total component analysis on determined metabolizable energy values for rations and feed ingredients[1]

Analysis	a	b_{yx}	Prediction equation
Fehlings: rations	-0.2972	0.0402	Y = -0.2972 + 0.0402X
Fehlings: ingredients	0.3618	0.0331	Y = 0.3618 + 0.0331X
Fehlings: combination	0.1814	0.0348	Y = 0.1814 + 0.0348X
Glucostat: rations	0.7062	0.0322	Y = 0.7062 + 0.0322X
Glucostat: ingredients	1.5065	0.0207	Y = 1.5065 + 0.0207X
Glucostat: combination	1.3693	0.0230	Y = 1.3693 + 0.0230X

[1]Values reported on a dry-matter basis.

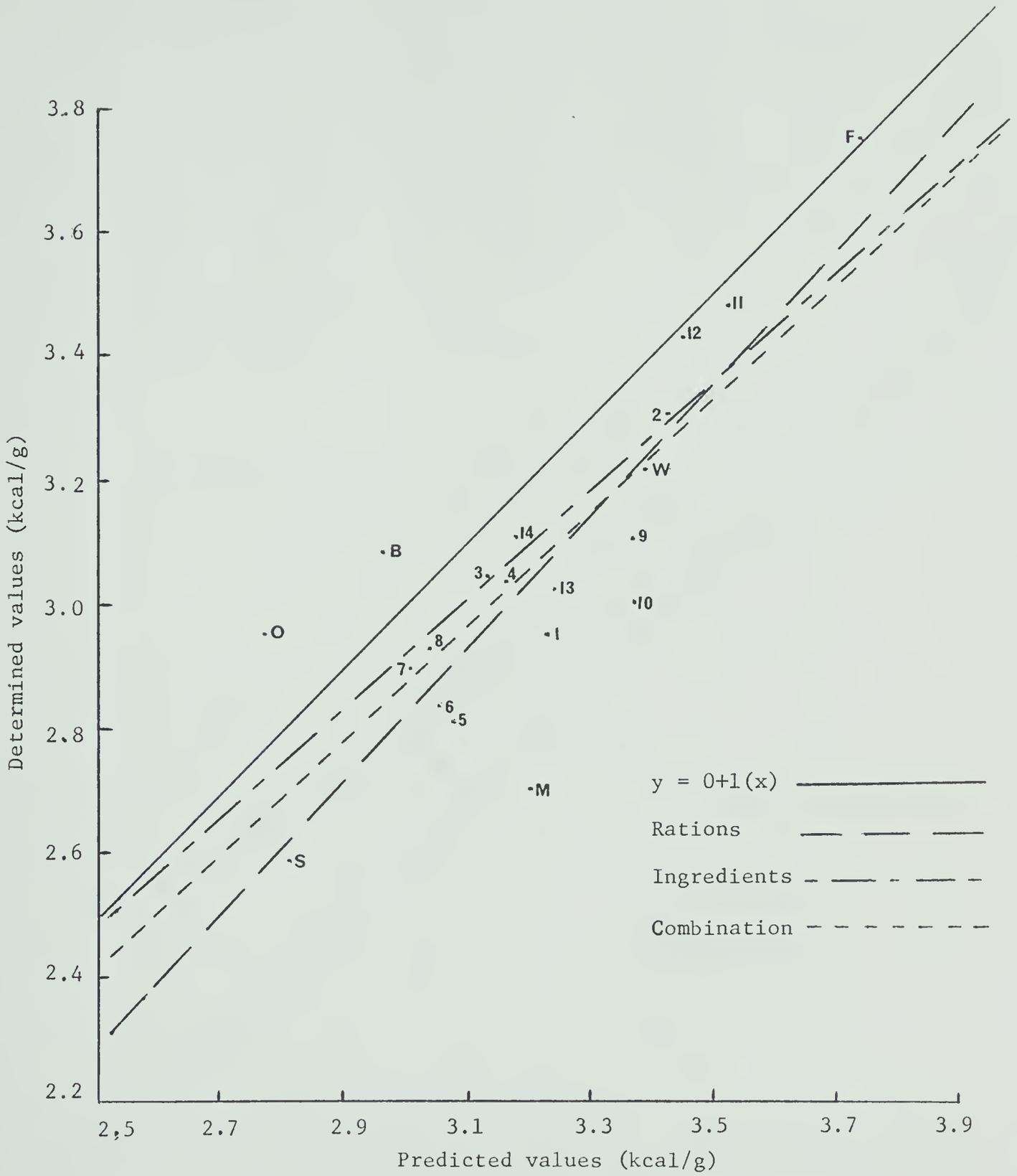

Figure 1. Relationship between predicted metabolizable energy values based on Fehlings analysis and biologically determined metabolizable energy values. Rations are numbered and feed ingredients are lettered.

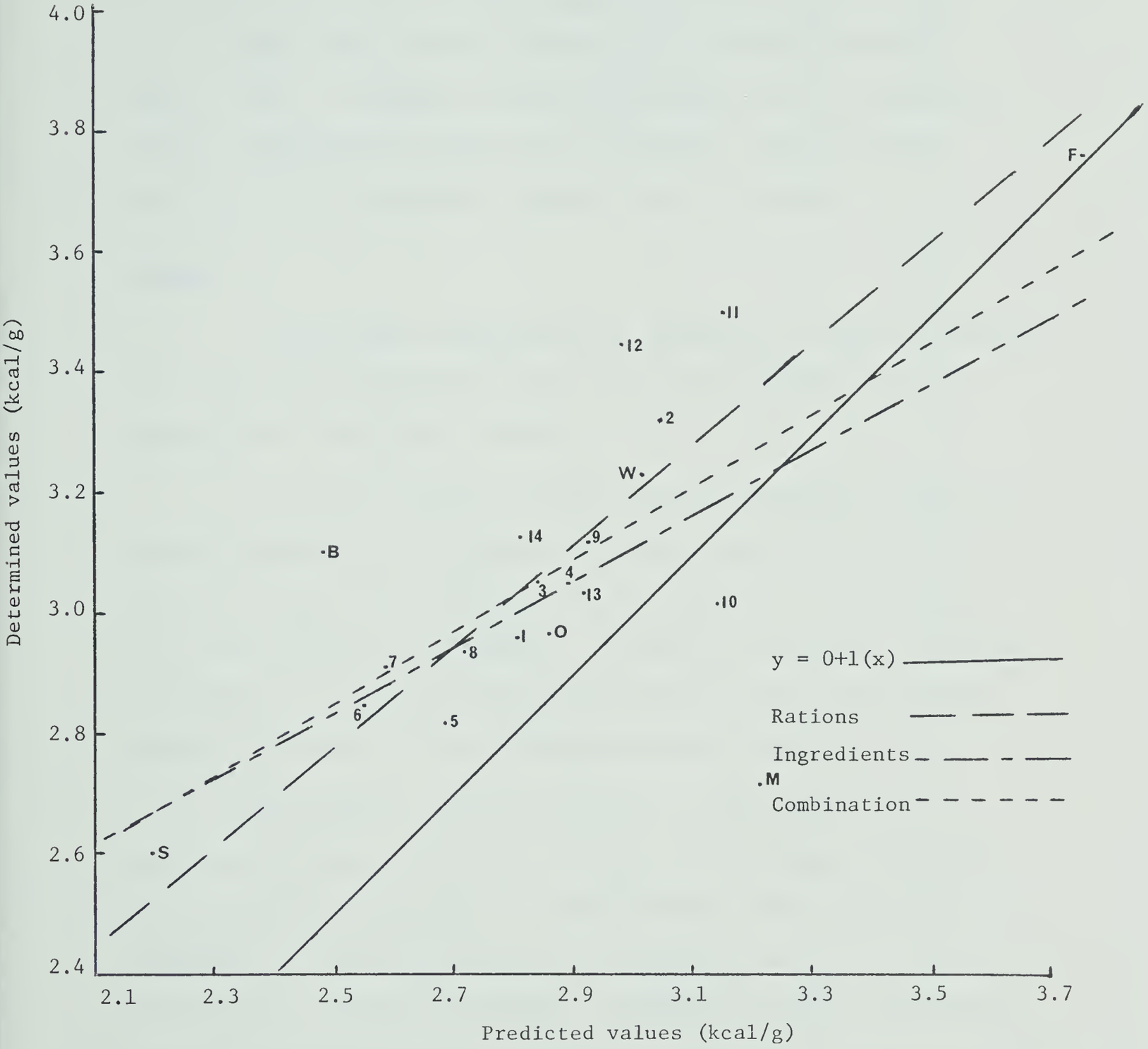

Figure 2. Relationship between predicted metabolizable energy values based on Glucostat analysis and biologically determined metabolizable energy values. Rations are numbered and feed ingredients are lettered.

In all cases, the prediction of M.E. content of rations was more accurate than that of single ingredients. This was probably due to the averaging effect of mixing ingredients since the M.E. content of the various energy-yielding components vary between feedstuffs (Halnan, 1951). Carpenter and Clegg (1956) mentioned this possibility and also postulated that there may be components other than those measured that may contribute a variable source of energy.

Summary

An experiment was conducted to determine the degree of accuracy with which the metabolizable energy content of a feedstuff could be predicted from its chemical analysis.

A prediction equation based on chemical analysis for protein, available carbohydrate and ether extract was used to calculate the M.E. content of rations and feedstuffs and the values found were compared with those determined biologically. Two methods of analysis of available carbohydrate, one using Fehlings reagent and the other using Glucostat reagent, were tested. The following results were obtained:

1. The prediction equation was found to yield correlation coefficients between M.E. values predicted using Fehlings reagent and those determined biologically for mixed rations, feed ingredients and a combination of mixed rations and feed ingredients of 0.87, 0.79 and 0.81, respectively. The Glucostat method of measuring available carbohydrate yielded less accurate results; correlation coefficients between predicted and biologically determined M.E. values for mixed rations, feed ingredients and a combination of the two were 0.75, 0.72 and 0.71, respectively.

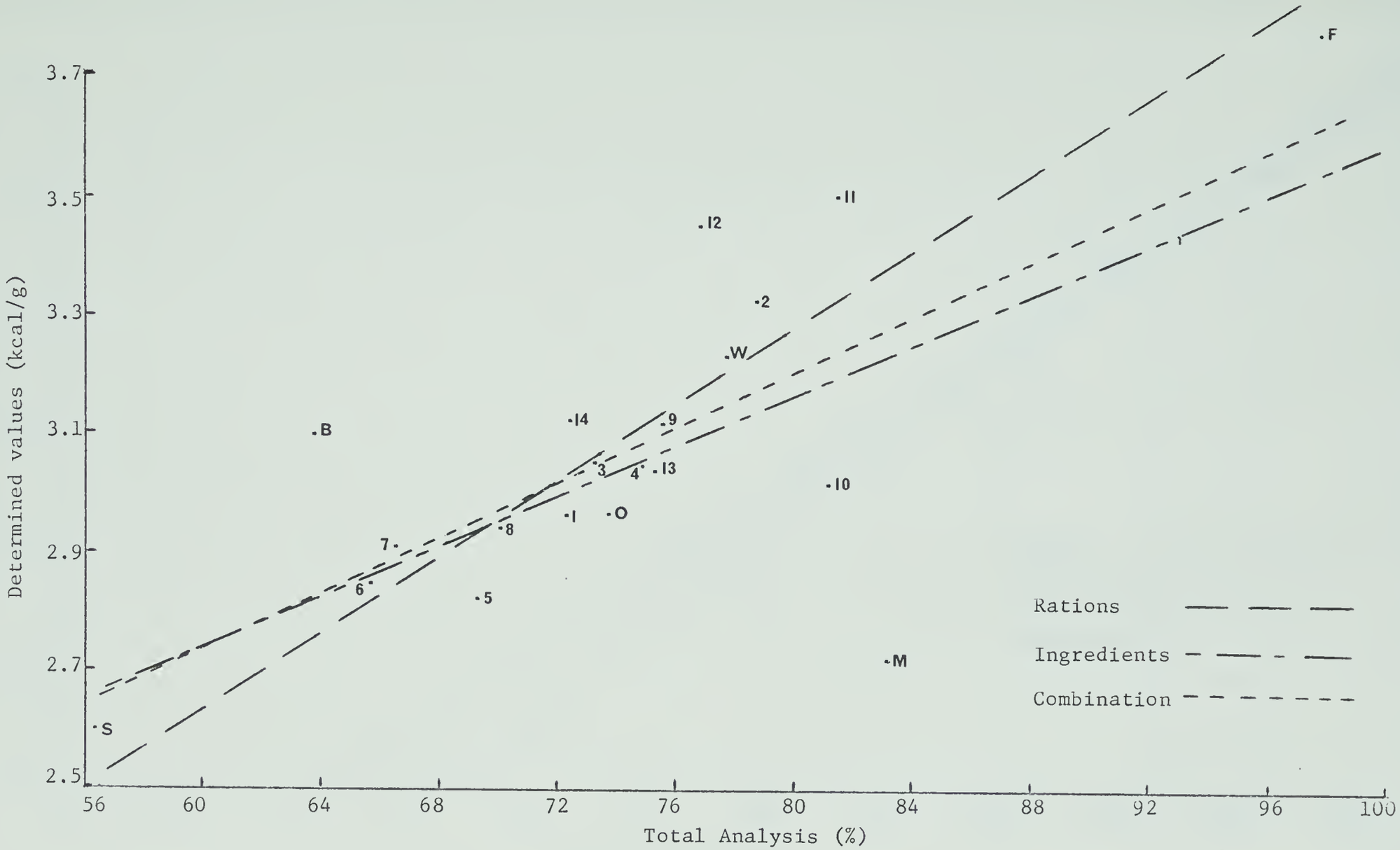

Figure 3. Relationship between determined metabolizable energy values and total analysis based on Glucostat analysis. Rations are numbered and feed ingredients are lettered.

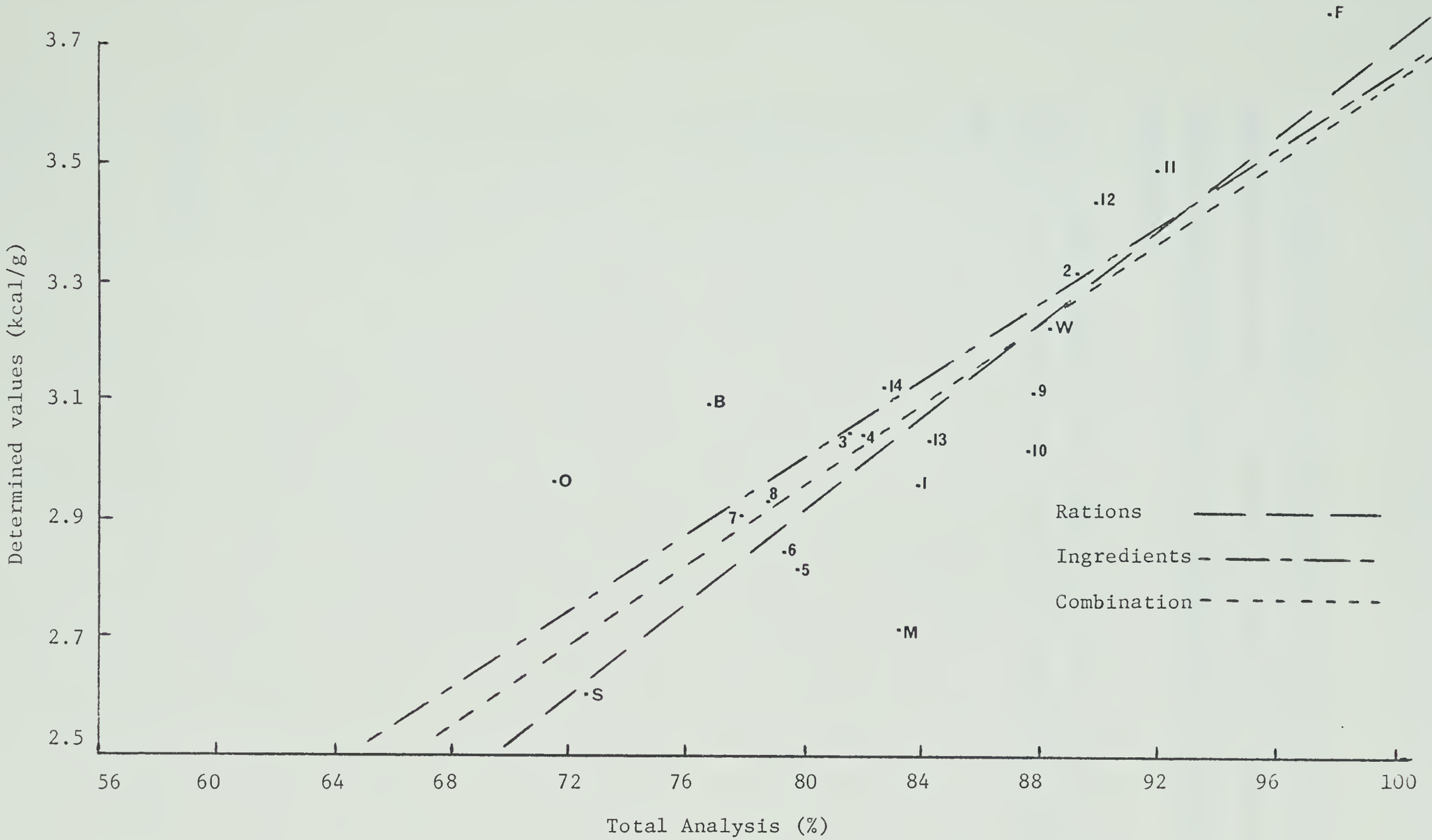

Figure 4. Relationship between predicted metabolizable energy values and total analysis based on Fehlings analysis. Rations are numbered and feed ingredients are lettered.

2. Use of averages analyses yielded estimates of M.E. for feed ingredients and rations of less accuracy than M.E. values predicted using either analytical method for determination of available carbohydrate.

3. Metabolizable energy values predicted for mixed rations were invariably more accurate than M.E. values predicted for single ingredients.

II. Substitution of Cereal Grains in Chicken Rations on a Nutrient-Equivalent Basis

A. Substitution of Grains Using Determined Metabolizable Energy Values.

Status of the Problem

The relative cost and availability of different cereal grains tends to vary from year to year and from area to area. It would therefore be useful if varying proportions of grains could be used in a ration without affecting its nutritive quality. This concept has become more important with the adoption of computer technology which allows more frequent adjustment of ration formulae as well as more accurate formulation based on the nutrient composition of the ingredients. At present, freedom in selection of ingredients must be restricted since the extent to which one grain may be substituted for another without affecting performance is not known. Thus, restrictions are only vaguely defined and full advantage cannot be taken of changes in price or availability of grain. Since it would be of considerable value if more concrete guidelines for the substitution of grains in poultry rations could be established, an experiment was undertaken to determine the extent to which wheat, oats and barley may be substituted for each other in broiler rations for chickens on a nutrient-equivalent basis without affecting performance.

Experimental

Two hundred and forty, day-old, male chicks (Dominant White × White Plymouth Rock) were divided into 16 comparable groups of 15 birds each. Two additional chicks were identified and randomly allotted to each group to be used in case any mortality occurred during the first

week of the experiment. If no mortality had occurred by one week of age, the birds that had been added to the group were removed; if mortality occurred in the group, the added birds replaced those that died.

The starting and finishing rations used in this experiment are shown in Tables 8 and 9. The rations were formulated to be isocaloric and isonitrogenous using determined values for metabolizable energy (chick bioassay) and protein (Experiment I). The rations used permitted study of the effects of single grains or combinations of grains on chick performance. A positive control (ration 8) was included for comparative purposes. Protein and M.E. levels of the control ration were not adjusted to correspond to the experimental rations.

Two groups of birds were allotted to each experimental ration and all groups were randomized as to location in the brooder house. The groups were housed in radiant-heated floor pens using straw as litter. Feed, water and insoluble grit were supplied ad libitum. The experiment was terminated at the end of nine weeks.

All chicks were wing-banded and group-weighed at the beginning of the experiment and at 2, 4 and 8 weeks of age. The chicks were weighed individually at 6 and 9 weeks of age. Feed consumption was recorded.

The data obtained at 6 and 9 weeks of age were subjected to analysis of variance on an IBM/360 model 67 computer using the One Way ANOVA2 program (Smillie, 1969). When significant differences were found, Duncans new multiple range test (Steele and Torrie, 1960) was employed.

Results and Discussion

The average weights of the chickens on the various treatments during the experiment (Table 10) indicated remarkably similar growth patterns for all treatments. No significant differences ($p < 0.05$) were found between rations with respect to average weight at 6 or 9 weeks of age.

The effect of treatment on feed conversion is also presented in Table 10. No significant differences were noted between treatments at 6 weeks of age but differences ($p < 0.025$) were observed at 9 weeks of age. When barley was used as the only source of grain (Ration 3) the amount of feed required per unit of gain was increased. Since the M.E. content of a ration is the primary factor in determining efficiency of feed utilization, it appeared that barley, when fed as the sole grain, yielded performance results which were not consistent with the determined M.E. content of the grain. No explanation for the difference noted can be advanced. The suggestions of Arscott et al. (1960) that an inhibitory substance in barley was responsible for the lower utilization and by Fry et al. (1958) that the carbohydrate portion of barley was only partially available did not appear to be applicable because determined M.E. values were used in formulating the rations.

With the exception of the control ration and the ration containing barley as the only grain, there were no significant differences between rations with respect to 9 week feed conversion values. The improved feed conversion obtained on the control ration (ration 8) would be expected on the basis of its higher M.E. content.

The results of the experiment reported herein demonstrated

Table 8. Composition of starter rations. Experiment II(A).

Ingredients	Ration number							
	1	2	3	4	5	6	7	8
	kg	kg	kg	kg	kg	kg	kg	kg
Ground wheat	73.0	-	-	33.0	32.5	-	21.0	61.615
Ground oats	-	60.5	-	33.0	-	30.0	21.0	-
Ground barley	-	-	59.0	-	32.5	30.0	21.0	-
Wheat shorts	1.065	1.065	0.715	1.265	1.145	0.665	1.535	-
Stabilized animal tallow	-	5.55	5.81	3.00	3.20	5.65	4.02	5.00
Dehydrated alfalfa meal	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Meat meal (55% protein)	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Herring meal (72% protein)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.0
Soybean meal (44% protein)	10.4	17.35	18.94	14.20	15.12	18.15	15.91	19.0
Ground limestone	0.75	0.75	0.75	0.75	0.75	0.75	0.75	1.0
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	-
Iodized salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.35
Manganese sulphate	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Zinc oxide	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Vitamin premix (1)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Calculated analysis								
Protein (%)	21.2	21.2	21.2	21.2	21.2	21.2	21.2	22.7
Metabolizable energy	2798	2798	2798	2798	2798	2798	2798	3133

[1] Supplied the following levels per kg of ration:
Vitamin A palmitate, 4950 I.U.; Vitamin D_3, 1650 I.C.U.; d-alpha-tocopherol acetate, 22 I.U.; menadione sodium bisulphite, 2.2 mg.; calcium pantothenate, 22 mg.; riboflavin, 6.6 mg.; niacin, 44 mg.; choline chloride, 374 mg.; vitamin B_{12}, 0.0132 mg.; folic acid, 2.17 mg.; procaine penicillin G 8.8 mg.; dl-methionine, 500 mg.

Table 9. Composition of finisher rations. Experiment II(A).

Ingredients	Ration Number 1	2	3	4	5	6	7	8
	kg	kg	kg	kg	kg	kg	kg	kg
Ground wheat	80.5	-	-	36.5	36.0	-	23.5	70.615
Ground oats	-	66.5	-	36.5	-	33.0	23.5	-
Ground barley	-	-	64.5	-	36.0	33.0	23.5	-
Wheat shorts	3.125	3.335	3.275	3.065	2.865	2.805	2.625	-
Stabilized animal tallow	-	6.10	6.43	3.35	3.54	6.26	4.44	5.00
Dehydrated alfalfa meal	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Meat meal (55% protein)	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Herring meal (72% protein)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.0
Soybean meal (44% protein)	0.84	8.53	10.26	5.05	6.06	9.40	6.90	10.0
Ground limestone	0.75	0.75	0.75	0.75	0.75	0.75	0.75	1.0
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	-
Iodized salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.35
Manganese sulphate	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Zinc oxide	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Vitamin premix(1)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Calculated analysis								
Protein (%)	18.4	18.4	18.4	18.4	18.4	18.4	18.4	20.13
Metabolizable energy (kcal)	2834	2834	2834	2834	2834	2834	2834	2994

[1]Supplied the following levels per kg of ration: Vitamin A palmitate, 4950 I.U.; vitamin D_3, 1650 I.C.U., d-alpha-tocopherol acetate, 22 I.U.; menadione sodium bisulphite, 2.2 mg.; calcium pantothenate, 22 mg.; riboflavin, 6.6 mg.; niacin, 44 mg.; choline chloride, 374 mg.; vitamin B_{12}, 0.0132 mg.; folic acid, 2.17 mg.; procaine penicillin G. 8.8 mg.; dl-methionine, 500 mg.

Table 10. Mean chick weights and feed conversion in Experiment II (A)[1]

Ration	Grains[2] used	Average weight					Feed conversion	
		2 wk.	4 wk.	6 wk.	8 wk.	9 wk.	6 wk.	9 wk.
		g	g	g	g	g	g feed/g gain	
1	W	160.4	436.5	831.6	1294.6	1511.4	1.78	2.26[ab]
2	O	149.3	406.3	789.3	1331.5	1508.2	1.77	2.30[b]
3	B	148.8	423.0	810.3	1220.3	1485.2	1.90	2.52[c]
4	W O	138.1	384.5	798.4	1249.0	1504.3	1.68	2.31[b]
5	W B	159.4	433.5	817.8	1313.2	1554.6	1.75	2.24[ab]
6	O B	145.9	416.0	774.2	1272.4	1491.0	1.84	2.38[bc]
7	W O B	142.2	398.5	797.2	1296.0	1551.3	1.75	2.30[b]
8	control	142.6	392.0	758.2	1335.3	1542.0	1.82	2.10[a]

[1]Values followed by the same superscript letters are not significantly different ($P < 0.05$).

[2]W, O and B refer to wheat, oats and barley respectively.

that, except in the ration in which barley was used as the sole grain,wheat, oats and barley, used alone or in combination, have feeding values related to their nutrient composition and can be substituted for each other on a nutrient-equivalent basis with no effect on performance. The results of the studies also indicated that when barley supplied one-half or less of the grain content of the ration, feed conversion was consistent with that expected on the basis of determined M.E. values.

Summary

An experiment was conducted to determine the extent to which wheat, oats and barley could be substituted for each other in isocaloric and isonitrogenous rations of equal nutritive value and the effect that the substitution would have on rate of gain and feed efficiency. The results obtained indicated the following:

1. The use of wheat or oats as the only grain in a broiler ration and the use of various combinations of wheat, oats or barley had no significant effect on rate of growth or feed efficiency.

2. The use of barley as the sole grain had no effect on rate of growth of broilers but resulted in a significant decrease in feed conversion at nine weeks of age.

B. Substitution of Grains using Predicted Metabolizable Energy Values.

Status of the Problem

The results of the previous experiment indicated that isocaloric and isonitrogenous rations could be formulated using wheat, oats or barley or combinations of the grains without affecting rate of growth. Feed conversion was also unaffected except when barley was used as the only grain in the ration in which case efficiency of feed conversion was reduced. From a practical point of view, the use of biologically determined M.E. values in formulating rations presents a major difficulty because the procedure involved is time-consuming and expensive. It would therefore be desirable if M.E. values predicted from chemical analysis could be used in place of determined values. Consequently an experiment was conducted to study the performance of chicks fed rations containing the same combination of grains that had been fed previously, but using predicted values for M.E. to formulate the nutrient-equivalent rations.

Experimental

The experimental procedure followed was the same as that used in Experiment II(A) except that the chicks were housed in electrically heated batteries with raised screen floors. The chicks were kept in the starting batteries until they were 4 weeks of age at which time they were transferred to growing batteries.

In the experiment 288,day-old, male chicks (Dominant White × White Plymouth Rock) were divided into 24 comparable groups of 12 birds each. Three groups of chicks were allotted at random to each

treatment.

The rations used in the experiment are shown in Tables 11 and 12. All rations, with the exception of the control (Ration 8), were formulated to be approximately isocaloric and isonitrogenous. Feed ingredients were analysed for protein and fat as outlined previously (Experiment 1). The M.E. content of the ingredients used in the experimental rations was predicted using the prediction equation based on the Glucostate carbohydrate analysis (Table 7). The following prediction equation was used: $y = 1.3693 + 0.0230\ X$.

Procedures used for analysis of the data were the same as those used in Experiment II(A).

Results and Discussion

Data on chick weights and feed conversion are shown in Table 13. The results obtained indicated that ration treatment had no significant effect ($P < 0.05$) on rate of gain at 6 or 9 weeks of age. Efficiency of feed conversion was likewise unaffected by treatment except when barley was used as the only grain in the ration. In this case (Ration 3) a significant reduction in feed efficiency was noted at 6 and 9 weeks of age. When barley supplied up to 50% of the grain portion of the ration (Rations 5, 6 and 7) feed conversion was apparently not affected in any way. The results closely paralleled those observed in Experiment II(A) although the effect on feed conversion of using barley as the only grain in the ration was apparent at 6 weeks as well as 9 weeks of age. With this exception it appeared that wheat, oats and barley could be substituted for each other on a nutrient-equivalent basis with no significant effect on performance.

Table 11. Composition of starter rations. Experiment II(B).

Ingredients	Ration number							
	1	2	3	4	5	6	7	8
	kg	kg	kg	kg	kg	kg	kg	kg
Ground wheat	70.5	-	-	32.5	32.9	-	21.3	61.615
Ground oats	-	60.7	-	32.5	-	30.5	21.3	-
Ground barley	-	-	61.6	-	32.9	30.5	21.3	-
Wheat shorts	0.565	0.565	0.565	0.665	0.565	0.665	0.665	-
Stabilized animal tallow	-	3.6	2.5	2.0	1.3	3.1	2.1	5.0
Dehydrated alfalfal meal	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Meat meal (55% protein)	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Herring meal (72% protein)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.0
Soybean meal (44% protein)	13.4	19.6	19.8	16.8	16.8	19.7	17.8	19.0
Ground limestone	0.75	0.75	0.75	0.75	0.75	0.75	0.75	1.0
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	-
Iodized salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.35
Manganese sulphate	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Zinc oxide	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Vitamin premix (1)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Calculated analysis								
Protein (%)	22.25	22.25	22.25	22.25	22.25	22.25	22.25	22.51
Metabolizable energy (kcal)	2687	2708	2691	2701	2688	2702	2694	2893

[1] Supplied the following levels per kg of ration: vitamin A, 4950 I.U.; vitamin D_3, 1650 I.C.U.; d-alpha-tocopherol acetate, 22 I.U.; menadione sodium bisulphite, 2.2 mg.; calcium pantothenate, 22 mg.; riboflavin, 6.6 mg.; niacin, 44 mg.; choline chloride, 374 mg.; vitamin B_{12}, 0.0132 mg.; folic acid, 2.17 mg.; procaine penicillin G, 8.8 mg.; dl-methionine, 500 mg.

Table 12. Composition of finisher rations. Experiment II(B).

Ingredients	Ration number 1	2	3	4	5	6	7	8
	kg	kg	kg	kg	kg	kg	kg	kg
Ground wheat	80.2	-	-	37.1	37.2	-	24.0	70.615
Ground oats	-	69.0	-	37.1	-	34.2	24.0	-
Ground barley	-	-	70.3	-	37.2	34.2	24.0	-
Wheat shorts	0.765	0.665	0.565	0.565	1.065	2.065	1.665	-
Stabilized animal tallow	-	4.2	2.8	2.3	1.6	3.6	2.5	5.0
Dehydrated alfalfa meal	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Meat meal (55% protein)	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Herring meal (72% protein)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.0
Soybean meal (44% protein)	3.5	10.6	10.8	7.4	7.4	10.4	8.3	10.0
Ground limestone	0.75	0.75	0.75	0.75	0.75	0.75	0.75	1.0
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	-
Iodized salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.35
Manganese sulphate	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Zinc oxide	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Vitamin premix (1)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Calculated analysis								
Protein (%)	19.33	19.33	19.33	19.33	19.33	19.33	19.33	19.99
Metabolizable energy (kcal)	2721	2750	2725	2739	2726	2739	2732	2925

[1] Supplied the following levels per kg of ration: vitamin A, 4950 I.U., vitamin D_3, 1650 I.C.U.,; d-alpha-tocopherol acetate, 22 I.U.; menadione sodium bisulphite, 2.2 mg; calcium pantothenate, 22 mg.; riboflavin, 6.6 mg.; niacin, 44 mg.; choline chloride, 374 mg.; vitamin B_{12}, 0.0132 mg.; folic acid, 2.17 mg.; procaine penicillin G, 8.8 mg.; dl-methionine, 500 mg.

The close similarity between the results obtained in Experiments II(A) and II(B) indicated that M.E. values predicted using the value for available carbohydrate based on analysis with Glucostat yielded performance results comparable to those obtained when biologically determined M.E. values were used. It thus appeared that the Glucostat-based prediction equation was capable of more accurate predictions of M.E. content of feedstuffs than was first indicated. A possible explanation is that the prediction equation used in this experiment had been derived from analytical data from Glucostat analysis whereas previously (Experiment 1) it had been based on the Fehlings analysis used by Bolton (1960). Since the Glucostat analysis for available carbohydrate apparently measures different sugars than Fehlings analysis, it is logical that the prediction equation should be derived from determinations made using the Glucostat method for measuring available carbohydrate.

Summary

An experiment was conducted to study the effects of using predicted M.E. values for feed ingredients in formulating rations for broilers. The experimental rations, which contained wheat, oats and barley, alone or in combination, were formulated to be approximately isocaloric and isonitrogenous. The data obtained in the feeding trial indicated the following:

1. Performance of broilers fed rations formulated using M.E. values predicted from chemical analyses was closely comparable to that which was obtained when biologically determined M.E. values were used.

TABLE 13. Mean chick weights and feed conversion in Experiment II(B)[1].

Ration	Grains[2] used	Average weight					Feed conversion	
		2 wk.	4 wk.	6 wk.	8 wk.	9 wk.	6 wk.	9 wk.
		g	g	g	g	g	(feed/gain)	
1	W	162.6	477.5	851.6	1197.8	1461.9	2.30^{ab}	2.62^{a}
2	O	158.8	414.2	831.7	1280.0	1520.6	2.28^{ab}	2.73^{a}
3	B	165.1	449.3	824.3	1155.0	1372.4	2.50^{c}	3.00^{b}
4	W O	170.4	482.6	875.9	1327.7	1518.9	2.26^{a}	2.63^{a}
5	W B	158.6	488.1	856.0	1297.4	1511.1	2.29^{ab}	2.71^{a}
6	O B	158.2	425.1	771.9	1180.5	1400.6	2.40^{abc}	2.76^{a}
7	W O B	163.9	475.7	850.6	1292.7	1484.0	2.23^{a}	2.71^{a}
8	control	147.5	436.5	780.8	1191.5	1377.5	2.46^{bc}	2.66^{a}

[1]Values followed by the same superscript letters are not significantly different. ($P < 0.05$).

[2]W, O and B refer to wheat, oats and barley respectively.

2. Substitution of wheat, oats or combinations of wheat, oats or barley for the grain portion of the ration had no effect on rate of gain or feed conversion of broiler chickens at 6 or 9 weeks of age.

3. When barley was used as the only grain in broiler rations, rate of gain was not significantly affected but a significant reduction in efficiency of feed conversion was noted at 6 and 9 weeks of age.

GENERAL DISCUSSION

The experiments reported herein demonstrated the potential value of being able to predict the metabolizable energy content of a ration or feed ingredient from the chemical analysis of the feed. When determined M.E. values were used in substituting wheat, oats and barley in chicken rations it was found that, with the exception of when barley was used singly, the grains could be substituted on a nutrient-equivalent basis with no effect on performance. When predicted M.E. values were used the results obtained were similar to those that had been obtained with determined values. Since the procedures involved in predicting M.E. values are far less complex and time-consuming than the procedure for the biological determination of M.E, it would appear that this procedure might well be adopted in formulating experimental rations. Since the energy level of the ration is the principal factor determining level of feed intake by the bird, accurate prediction of M.E. would permit more accurate formulation of rations. This in turn should result in improved performance and decreased waste of ration components with an accompanying reduction in cost of production.

The equation used for the prediction of M.E. initially used in this study was derived by Carpenter and Clegg (1956) and was modified by use of the available carbohydrate analysis suggested by Bolton (1960). The analysis of available carbohydrate was in turn modified to compare two methods of measurement; Fehlings analysis and Glucostat analysis. The original analysis for available carbohydrate (Bolton, 1960) used Fehlings reagent and thus yielded a more accurate prediction

of M.E. than Glucostat analysis when used in conjunction with the equation of Carpenter and Clegg (1956). However, when a prediction equation was derived from analytical data from Glucostat analyses, a feeding trial demonstrated that the results obtained were generally equivalent to those noted when determined M.E. values were used.

It was apparent from these studies that barley was not completely comparable to the other grains on a nutrient-equivalent basis. While wheat, oats and combinations of wheat, oats and barley yielded performance results consistent with their nutrient composition, feed conversion was depressed when barley was the only grain used in an isocaloric-isonitrogenous ration. Although studies have been undertaken to determine the reason for this effect (Arscott et al., 1960; Frv et al., 1958) and hypotheses have been advanced, the reason for the depression is not clear.

BIBLIOGRAPHY

Anderson, D.L., and F.W. Hill. 1955. Determination of metabolizable energy values for chicks for pure carbohydrate, cellulose, fat and casein. Poultry Sci. 34:1176.

Anderson, D.L., F.W. Hill, and R. Renner. 1958. Studies of the metabolizable and productive energy of glucose for the growing chick. J. Nutrition. 65:561-574.

Arscott, G.H., R.J. Rose, and J.A. Harper. 1960. An apparent inhibitor in barley influencing efficiency of utilization by chicks. Poultry Sci. 39:268-270.

Association of Official Agricultural Chemists. 1960. Official methods of analysis. 9th edition. Washington, D.C.

Axelsson, J., and S. Eriksson. 1951. Determination of metabolizable energy in poultry feedstuffs and rations. Ninth Worlds Poultry Congress Proc.. 2:160-162.

Baldini, J.T. 1960. The effect of dietary deficiency on the energy metabolism of the chick. Poultry Sci. 39:1232.

Baldini, J.T. 1961. The effect of dietary deficiency on the energy metabolism of the chick. Poultry Sci. 40:1177-1183.

Begin, J.J. 1967. The relation of breed and sex of chickens to the utilization of energy. Poultry Sci. 46:379-383.

Biely, J., B.E. March, G.L. Inkin, and G.T. Eedy. 1951. Wheat versus corn in the Connecticut broiler ration. Poultry Sci. 30:593-598.

Bolton, W. 1954. The digestibility of the carbohydrate complex of bran and oats by adult cocks. Tenth Worlds Poultry Congress Proc. pp. 94-98.

Bolton, W. 1960. The determination of soluble carbohydrates in poultry foods. Analyst. 85:189-192.

Carew, L.B. Jr., and F.W. Hill. 1961. Effect of methionine deficiency on the utilization of energy by the chick. J. Nutrition. 74:185-190.

Carpenter, K.J., and K.M. Clegg. 1956. The metabolizable energy of poultry feeding stuffs in relation to their chemical composition. J. Sci. Food Agric. 7:45-51.

Carpenter, K.J., and K.M. Clegg. 1957. The relative value of three cereals as protein sources for growing chicken. Br. J. Nutr. 11:358-364.

Clegg, K.M. 1956. The application of the Anthrone reagent to the estimation of starch in cereals. J. Sci. Food Agric. 7: 40-44.

Combs, G.F., and G.L. Romoser. 1955. A new approach to poultry feed formulation. Feed Age 5, No. 3, 50-58.

Cullen, M.P., O.G. Rasmussen, and O.H.M. Wilder. 1962. Metabolizable energy value and utilization of different types and grades of fat by the chick. Poultry Sci. 41:360-367.

Dansky, L.M., and F.W. Hill. 1951. Effect of energy level and physical nature of the diet on growth and body composition of chicks. Poultry Sci. 30: 910.

Davidson, J., J. Mathieson and R.B. Williams. 1962. The relative values of cereal proteins for chick growth. Br. J. Nutr. 16:551-557.

Davidson, J., I. McDonald, J. Mathieson, and R.B. Williams. 1961. Utilization of dietary energy by poultry. 2. Effects of indigestible organic matter and protein on the utilization of metabolizable energy for growth. J. Sci. Food Agric. 12: 425-439.

Davidson, J., I. McDonald, and R.B. Williams. 1957. The utilization of dietary energy by poultry. 1. A study of the algebraic method of determining the productive energy of poultry feeds. J. Sci. Food Agric. 8:173-182.

Donaldson, W.E., G.F. Combs, and G.L. Romoser. 1956. Studies on energy levels in poultry rations. 1. The effect of caloric: protein ratio of the ration on growth, nutrient utilization and body composition of chicks. Poultry Sci. 35:1100-1105.

Dymsza, H., R.V. Boucher, and M.G. McCartney. 1955. The influence of fibre content and physical form of the diet on the energy requirement of turkeys. 1. Studies with turkey poults. Poultry Sci. 34:435-439.

Fisher, H., and H.S. Weiss. 1956. Feed consumption in relation to dietary bulk and energy level: the effect of surgical removal of the crop. Poultry Sci. 35:418-423.

Foster, W.H. 1968. Variation within and between birds in the estimation of the metabolizable content of diets for laying hens. J. Agr. Sci. 71(2):153-159.

Fraps, G.S. 1946. Composition and productive energy of poultry feeds and rations. Texas Agr. Exp. Sta. Bulletin 678.

Fraps, G.S., and E.C. Carlyle. 1939. Utilization of energy of wheat products by chickens. J. Nutrition. 18:385-398.

Fry, R.E., J.B. Allred, L.S. Jensen, and J. McGinnis. 1958. Influence of enzyme supplementation and water treatment on the nutritional value of different grains for poults. Poultry Sci. 37:372-375.

Halnan, E.T. 1951. The nutritive (energy) value of poultry feeds. Ninth Worlds Poultry Congress Proc. 2:3-6.

Henneberg, W., and F. Stohmann. Cited by Bolton, W. 1954. The digestibility of the carbohydrate complex of bran and oats by adult cocks. Tenth Worlds Poultry Congress Proc. pp. 94-98.

Heuser, G.F., L.C. Norris, H.T. Peeler, and M.L. Scott. 1944. Further studies on the apparent effect of digestibility upon growth, weight-maintenance and egg production. Poultry Sci. 24: 142-145.

Hill, F.W., and D.L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with chicks. J. Nutriton. 64:587-603.

Hill, F.W., D.L. Anderson, and L.M. Dansky. 1956. Studies of the energy requirements of chickens. 3. The effect of dietary energy levels on the rate and gross efficiency of egg production. Poultry Sci. 35:54-59.

Hill, F.W., D.L. Anderson, R. Renner, and L.B. Carew, Jr. 1960. Studies of the metabolizable energy of grain and grain products for chickens. Poultry Sci. 39:573-579.

Hill, F.W., and L.M. Dansky. 1950. Studies on the protein requirements of chicks and its relationship to dietary energy levels. Poultry Sci. 29:763.

Hill, F.W., and L.M. Dansky. 1954. Studies of the energy requirements of chickens. 1. The effect of dietary energy level on growth and feed consumption. Poultry Sci. 33:112-119.

Hoppner, K.H., B.D. Owen and F.W. Sosulski. 1968. Proximate principles and B-vitamins in Saskatchewan feed grains. Can. J. Anim. Sci. 48(2):135-143.

Kalmbach, M.P., and L.M. Potter. 1959. Studies in evaluating energy content of feed for chicks. Poultry Sci. 38:1217.

Lockhart, W.C., R.L. Bryant, and D.W. Bolin. 1966. A comparison of several methods of determining the metabolizable energy content of Durum wheat and wheat cereal by chicks. Poultry Sci. 46: 805-810.

Matterson, L.D., L.M. Potter, L.D. Stinson and E.P. Singsen. 1955. Studies on the effect of varying protein and energy level in poultry rations on growth and feed efficiency. Poultry Sci. 34:1210.

Matterson, L.D., L.M. Potter, M.W. Stutz, and E.P. Singsen. 1965. The metabolizable energy of feed ingredients for chickens. Univ. of Conn. Agric. Exp. Sta. Research Report 7.

Maw, W.A. 1939. Cereals in the fattening ration. Seventh Worlds Poultry Congress Proc. pp. 177-178.

Mraz, F.R., R.V. Boucher and M.G. McCartney. 1957. The influence of energy:volume ratio on growth response in chickens. Poultry Sci. 36:1217-1221.

Olson, G., W.C. Lockhart, D.W. Bolin and R.L. Bryant. 1961. Metabolizable energy values of soybean oil meal and meat meal as affected by protein level and type. Poultry Sci. 40:260-262.

Ozment, D.D., K.E. Dunkelgod,L.V.Tonkinson, E.W. Gleaves, R.H. Thayer and F.F. Davies. 1962. Comparing milo and corn in broiler diets on an equivalent nutrient intake basis. Poultry Sci. 42:472-481.

Panda, J.N., and G.F. Combs. 1950. Studies on the energy requirements of the chick for rapid growth. Poultry Sci. 29:774-775.

Peterson, D.W., G.R. Grau, and N.F. Peek. 1954. Growth and food consumption in relation to dietary levels of protein and fibrous bulk. J. Nutrition 52:241-257.

Potter, L.M., L.D. Matterson, A.W. Arnold, W.J. Pudelkiewicz, and E.P. Singsen. 1960. Studies in evaluating energy content of feeds for the chick. 1. The evaluation of the metabolizable energy and productive energy of alpha cellulose. Poultry Sci. 39:1166-1178.

Potter, L.M., W.J. Pudelkiewicz, L. Webster, and L.D. Matterson. 1962. Metabolizable energy and digestibility evaluation of fish meal for chickens. Poultry Sci. 41:1745-1752.

Richardson, C.E., A.B. Watts, and E.A. Epps. 1958. The effect of added fibre with and without fat in a practical broiler ration. Poultry Sci. 37:1278-1283.

Scott, H.M., L.D. Matterson and E.P. Singsen. 1947. Nutritional factors influencing growth and efficiency of feed utilization. 1. The effect of the source of carbohydate. Poultry Sci. 26:554.

Sibbald, I.R., J. Czarnocki, S.J. Slinger and G.C. Ashton. 1962. Relationships between chemical composition and the metabolizable energy content of poultry feedstuffs. Poultry Sci. 41:1682.

Sibbald, I.R., J. Czarnocki, S.J. Slinger and G.C. Ashton. 1963. The prediction of metabolizable energy content of poultry feedingstuffs from a knowledge of their chemical composition. Poultry Sci. 42:486-492.

Sibbald, I.R., and S.J. Slinger. 1962a. Factors affecting the metabolizable energy content of poultry feeds. 10. A study of the effect of level of dietary inclusion on the metabolizable energy values of several high protein feeding stuffs. Poultry Sci. 41:1282-1289.

Sibbald, I.R., and S.J. Slinger. 1962b. The metabolizable energy of materials fed to growing chicks. Poultry Sci. 41:1612-1613.

Sibbald, I.R., and S.J. Slinger. 1962c. The relationship between classical and corrected metabolizable energy values. Poultry Sci. 41:1007-1009.

Sibbald, I.R., and S.J. Slinger. 1963a. Nutritive values of ten samples of Western Canadian grains. Poultry Sci. 42:276-277.

Sibbald, I.R., and S.J. Slinger. 1963b. A biological assay for metabolizable energy in poultry feed ingredients together with findings which demonstrate some of the problems associated with the evaluation of fats. Poultry Sci. 42:313-325.

Sibbald, I.R., and S.J. Slinger. 1963c. Factors affecting the metabolizable energy content of poultry feeds. 12. Protein quality. Poultry Sci. 42:707-710.

Sibbald, I.R., S.J. Slinger, and G.C. Ashton. 1960. The weight gain and feed intake of chicks fed a ration diluted with cellulose or kaolin. J. Nutrition. 72:441-446.

Sibbald, I.R., S.J. Slinger, and W.F. Pepper. 1962. Interrelationships between riboflavin, niacin, vitamin B_{12} and methionine measured in terms of chick weight gains and dietary metabolizable energy values. Poultry Sci. 41:380-386.

Sibbald, I.R., J.D. Summers, and S.J. Slinger. 1959. Factors affecting the metabolizable energy content of poultry feeds. Poultry Sci. 38:1247.

Sibbald, I.R., J.D. Summers, and S.J. Slinger. 1960. Factors affecting the metabolizable energy content of poultry feeds. Poultry Sci. 39:544-556.

Slinger, S.J., I.R. Sibbald, and W.F. Pepper. 1964. The relative abilities of two breeds of chickens and two varieties of turkeys to metabolize dietary energy and dietary nitrogen. Poultry Sci. 43:329-333.

Steele, R.G.D., and J.H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Company, Inc., New York.

Sunde, M.L. 1956. A relationship between protein levels and energy level in chick rations. Poultry Sci. 35:350-354.

Smillie, K.W. 1969. STATPACK 2: An APL statistical package. Pub. No. 17. Dept. of Computing Science, University of Alberta, Edmonton, Alberta.

Titus, H.W., A.L. Mehring Jr., D. Johnson Jr., L.L. Nesbitt, and T. Tomas. 1959. An evaluation of M.C.F. (Micro-Cel-Fat), a new type of fat product. Poultry Sci. 38:1114-1119.

Vohra, P. 1966. Energy concepts for poultry nutrition. World's Poultry Sci. J. 22:6-24.

Waldroup, P.W., D.E. Greene, R.H. Harris, J.F. Maxey, and E.L. Stephenson. 1967. Comparison of corn, wheat, and milo in turkey diets. Poultry Sci. 46:1581-1585.

Zelenka, J. 1968. Influence of age of chicken on the metabolizable energy values of poultry diets. Brit. Poultry Sci. 9: 135-142.

APPENDIX A

Metabolizable Energy Determination

The method used was similar to that reported by Sibbald and Slinger (1963b).

Procedure

Chromic oxide was mixed in the assay rations at a level of 0.3% to serve as an index substance.

Fecal samples from each experimental treatment were collected during the last three days of the experimental period. The samples were placed in plastic bags and stored in a freezer at -20°F. Prior to analysis the three samples from each treatment were pooled and homogenized with distilled water to give a thick slurry. The pH of the samples was lowered to 5.7 by addition of 6 M H_2SO_4 . The samples were dried in a ventilated oven at 60°C and ground to pass a 20 mesh screen. Rations for analysis were also ground to pass a 20 mesh screen and were dried in a vacuum oven at 90°C for one hour.

The dried and ground samples of rations and excreta were assayed for gross energy using a Parr Oxygen Bomb Calorimeter. The feed : excreta chromic oxide ratios were determined by the method of Hill and Anderson (1958). Nitrogen determinations were conducted by the method of Kjeldahl (AOAC, 1960).

Prior to calculating the M.E. content of the test ingredients, it was necessary to derive the M.E. values of the rations fed. Classical M.E. values for the assay rations fed were calculated as follows:

M.E. per gram of feed = Gross energy per gram of feed -

$$\left(\frac{Cr_2O_7/g \text{ of feed}}{Cr_2O_7/g \text{ of excreta}} \times \text{Gross energy per gram of excreta} \right)$$

Metabolizable energy values corrected for nitrogen retention were calculated as follows:

Corrected M.E./g of feed = Classical M.E./g of feed -

$$\left[\text{Gross nitrogen/g of feed} - \left(\frac{Cr_2O_7/g \text{ of feed}}{Cr_2O_7/g \text{ of excreta}} \times \right.\right.$$

$\left.\left.\text{Gross nitrogen/g of excreta}\right)\right] \times 8.73$. The factor 8.73 represents the value derived by Titus et al. (1959) for the combustible energy of urine per gram of nitrogen.

When the M.E. values for the assay rations were obtained, it was possible to derive the M.E. values for the ingredients being assayed. Since 41.35 g of vitamin - mineral premix was added to each kg of assay ration, it was necessary to multiply the ration M.E. values by 1.04135 to obtain the M.E. values for the basal ration plus the substituted test ingredient. This contributed a small but constant error of insignificant proportion.

Using the corrected M.E. value for the rations it was then possible to calculate the M.E. values of the test ingredients by solving all possible pairs of simultaneous equations for the rations containing the ingredient in question as follows:

The rations consist of varying proportions of basal (B) and assay ingredient (A).

100B + 0A = determined M.E.

50B + 50A = determined M.E.

40B + 60A = determined M.E.

This allows for the solution of 3 pairs of simultaneous equations resulting in 3 estimations of the M.E. of each test ingredient which are then averaged. Since every treatment was replicated, each estimation of M.E. of test ingredients was based on the results of 6 treatments.

APPENDIX B

Available Carbohydrate Determination

The method followed was similar to that reported by Bolton (1960).

Theory

Analysis of available carbohydrate entailed the enzymatic conversion by Takadiastase[1] of available carbohydrate to maltose and glucose; maltose was then hydrolysed to glucose with sulfuric acid.

Procedure

1. Weigh a one gram sample of ration or feed ingredient into a 100 ml flask.

2. Moisten the sample with a few drops of ethanol and add 20 ml of water.

3. Heat the contents with continuous swirling to gelatinize the starches.

4. Allow the flask to cool.

5. Add 0.5 ml of 5% (w/v) acetic acid and 0.1 gram of Takadiastase to the flask. Mix the contents and wash the walls with a minimum amount of water.

6. Add 0.5 ml of toluene, cap the flasks with aluminum foil and leave overnight.

7. Heat the incubated contents of the flasks to boiling and

[1] Sigma Chemical Company.

filter through a Whatman #3 filter paper in a Buchner funnel. Wash the residue twice with boiling water.

8. Dilute the filtrate to a volume of 190 ml, and clarify by addition of 5 ml of 5% (w/v) zinc sulphate solution and 5 ml of 3.67% (w/v) potassium ferrocyanide solution.

9. Filter the suspension through a 15 cm Whatman #1 filter paper.

10. Make 100 ml of the filtrate in a 250 ml flask approximately 1.5 N by the addition of 6 ml of 72% (w/v) H_2SO_4 . Cap the flask with aluminum foil and heat in steam for 2 hours.

11. When the solution is cool neutralize with about 14 ml of 40% (w/v) NaOH and dilute H Cl. Record the volume and determine the glucose content.

12. Available carbohydrate content equals "glucose content × 0.91".

Determination of Glucose

Glucose was determined using two methods. The first method of glucose determination was that used by Bolton (1960) using Fehlings reagent (AOAC, 1960) for estimation of reducing sugars present. The second method of glucose determination used was the colorimetric measurement of β-D-glucose using the Glucostat[2] enzyme reagent. Details of the Glucostat method are outlined below.

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[2]Worthington Biochemical Corporation.

Reagents

1. 2% $ZnSO_4.7H_2O$

2. 1.8% $Ba(OH)_2.8H_2O$

3. Glucostat reagent prepared as follows:

Add 60 ml of distilled water to a 100 ml graduated cylinder. Dissolve the chromagen in distilled water and add to the cylinder. Dissolve the contents of the Glucostat vial and add to the cylinder. Adjust the final volume to 80 ml.

4. Standard solution containing 100-300 mg of glucose per 100 ml of water.

Procedure

1. Add 0.2 ml of prepared sample to 1.8 ml of H_2O . Mix and add 1.0 ml of $Ba(OH)_2$ solution. Mix and add 1.0 ml of $ZnSO_4$ solution. After mixing, filter or centrifuge.

2. Set up a series of test tubes. Include one for each unknown, one for each reagent blank, and at least one for a standard.

3. Add 2.0 ml of filtrate or standard into the respective tubes. Into the reagent blank, add 2.0 ml of distilled water.

4. At timed intervals, add 8.0 ml Glucostat reagent and mix. Let stand 10 minutes at room temperature.

5. At the same timed intervals, add 1 drop of 4 N HCl to each and mix. Let stand at least 5 minutes. Color is stable for several hours.

6. Read absorbancy of solutions in a suitable photometer at 400 to 425 mu, set at zero with the reagent blank.

7. Calculation of glucose concentration is made according to the following equation:

$$\frac{Au}{As} \times Cs = Cu$$

where A = absorbancy

C = concentration of glucose

u = unknown

s = standard